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A BACTERIOLOGICAL TECHNIQUE FOR THE ISOLATION OF MARINE DIATOMS

THEODORE J. STARR[a]

These observations were noted during bacteriological studies of marine environments.

Samples of estuarine waters, sediments, and plankton tows were diluted quantitatively with sterile, aged sea water. Aliquots of each dilution, 50–500 ml., were filtered through 'millipore filter' membranes (Millipore Filter Corp., Bedford, Mass.). Filters were placed, right side-up, on the surface of petri-plates which contained a medium of the following composition: aged sea water to volume; Difco agar, 1.0 per cent; sodium acetate, 0.01 per cent; and yeast extract, trypticase, and peptone, 0.05 per cent each. The final pH before autoclaving at 15 lb. for 15 min. was pH 7.6–7.8. Cultures were incubated under a battery of fluorescent lights at 22° C.–25° C. for 1–3 wk. Using a binocular microscope with a magnification of 5×–10×, typical diatom tracts were observed as a network surrounding the filter and extending outward along the surface of the agar. Bacterial and algal colonies were confined to the edge or surface of the filter. Quite often, samples of sediments and plankton tows would contain agar-digesting bacteria which would liquefy the agar along the edge of the filter and inhibit the typical outward migration of the diatoms.

A fine platinum wire needle was used to cut small pieces of agar containing cells from the extreme edge of the diatom network. The agar blocks were transferred to sterile plates of the above composition and were incubated until diatom tracts were observed again. Usually best results were obtained by placing each agar block with the cell side in contact with the medium. In this manner, several serial transfers were made in order to insure culture purity. Sterility tests were made in test tubes of the same medium minus the agar.

Using this technique, several species of *Nitzschia* and *Navicula* were isolated and maintained in pure culture. Other genera were obtained on occasion but could not be successfully transferred more than 1 or 2 times in pure culture. This could be attributed to a number of causes including lack of essential nutrients. It is suggested that variation in the chemical composition of the isolation medium may facilitate the isolation of the more fastidious diatoms.

Other investigators have used the migration characteristic of microorganisms for cultures of amebae (1,2) and Myxomycetes (3). Provasoli and Pintner (4) described the rapid growth of the filaments of *Phormidium persicinum*, an auxotrophic marine red-pigmented blue-green alga, as a means of eliminating bacterial growth. Other workers (5) used antibiotics in their initial isolation medium. Watson (6) used the agar block technique for the isolation of *Labyrinthula*, the causative agent of wasting disease of *Zostera marina* (ell-grass). In most instances, isolations depended on the migratory ability of the organism.

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